

Appl. No. 09/938,112
Reply to Office action of July 9, 2004

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1-20. (Cancelled).

21. (Currently amended) A method for obtaining an agent for alleviating pain, the method comprising:

(a) producing a genetic construct having nucleic acids encoding a clostridial neurotoxin;

(b) incorporating the construct into a host cell;

(c) culturing the cell under conditions sufficient to express ~~expressing the construct to produce~~ the clostridial neurotoxin; and

(d) covalently attaching or recombinantly fusing the clostridial neurotoxin to a targeting moiety which comprises substance P, wherein H_c has been removed from the clostridial neurotoxin or modified so as to reduce the ability of the clostridial neurotoxin to bind to a receptor for the H_c at a neuromuscular junction.

22. (Original) The method of claim 21, wherein the covalent linkage includes one or more spacer components.

23-35. (Cancelled)

36. (Currently amended) A plasmid encoding a clostridial neurotoxin, comprising:

Appl. No. 09/938,112
Reply to Office action of July 9, 2004

(a) a first nucleotide sequence ~~region~~ comprising;
(i) a first ~~portion~~ nucleotide segment encoding an amino acid sequence ~~region~~ comprising a targeting moiety ~~that comprises of~~ substance P ~~and is~~ able to specifically bind to receptors on cells under physiological conditions; and (ii) a second ~~portion~~ nucleotide segment encoding an amino acid sequence ~~region~~ comprising a translocation element able to facilitate the transfer of a polypeptide across an endosome membrane; and

(b) a second nucleotide sequence ~~region~~ encoding an ~~additional~~ amino acid sequence ~~region~~ comprising a therapeutic element having an intracellular protease biological activity when released into the cytoplasm of a target cell, and an ~~origin of element for~~ replication directing plasmid replication by a host cell, wherein H_c has been removed from the clostridial neurotoxin or modified so as to reduce the ability of the clostridial neurotoxin to bind to a receptor for the H_c at a neuromuscular junction.

37. (Currently amended) A method of making a clostridial neurotoxin comprising:

(a) inserting the plasmid of claim 36 into a suitable host cell,

(b) ~~growing~~ culturing the host cell under conditions sufficient to express the clostridial neurotoxin in culture, and

(c) ~~permitting the host cell to express the polypeptide from the plasmid~~ isolating the clostridial neurotoxin.

38-66. (Cancelled)

Appl. No. 09/938,112
Reply to Office action of July 9, 2004

67. (Currently amended) A method for obtaining an agent for alleviating pain, the method comprising:

- (a) producing a genetic construct having nucleic acids encoding a clostridial neurotoxin;
- (b) incorporating the construct into a host cell;
- (c) ~~expressing the construct to produce~~ culturing the cell under conditions sufficient for expression of the clostridial neurotoxin; and
- (d) covalently attaching the expressed clostridial neurotoxin to substance P, wherein H_c has been removed from the clostridial neurotoxin or modified so as to reduce the ability of the clostridial neurotoxin to bind to a receptor for the H_c at a neuromuscular junction.

68. (Previously presented) The method of claim 67, further comprising covalently attaching at least one spacer component between the clostridial neurotoxin and the substance P.

69. (Currently amended) The method of claim 67, wherein the clostridial neurotoxin ~~is produced by~~ has an amino acid sequence substantially identical to a neurotoxin from an organism selected from the group consisting of Clostridial beratti, Clostridial butyricum, Clostridial botulinum, and Clostridial tetani.

70. (Currently amended) The method of claim 67, wherein the expressed clostridial neurotoxin ~~is~~ has an amino acid sequence substantially identical to a botulinum toxin selected from the group consisting of serotype A, serotype B, serotype C1, serotype D, serotype E, serotype F, and serotype G.

Appl. No. 09/938,112
Reply to Office action of July 9, 2004

71. (Currently amended) The method of claim 67, wherein the expressed clostridial neurotoxin ~~is~~ has an amino acid sequence substantially identical to botulinum toxin serotype A.

72. (Previously presented) The method of claim 67, wherein the clostridial neurotoxin comprises an H_N and an L chain.

73. (Currently amended) The method of claim 72, wherein the H_N is produced by a translocation domain of a clostridial neurotoxin having an amino acid sequence substantially identical to a clostridial neurotoxin from an organism selected from the group consisting of Clostridial beratti, Clostridial butyricum, Clostridial botulinum, and Clostridial tetani.

74. (Currently amended) The method of claim 72, wherein the L chain is produced by is a light chain of a clostridial neurotoxin having an amino acid sequence substantially identical to a clostridial neurotoxin from an organism selected from the group consisting of Clostridial beratti, Clostridial butyricum, Clostridial botulinum, and Clostridial tetani.

75. (Currently amended) The method of claim 72, wherein the H_N is obtained from a translocation domain having an amino acid sequence substantially identical to a translocation domain of a botulinum toxin selected from the group consisting of botulinum toxin serotype A, serotype B, serotype C1, serotype D, serotype E, serotype F, and serotype G.

76. (Cancelled)

Appl. No. 09/938,112
Reply to Office action of July 9, 2004

77. (Currently amended) A method for obtaining an agent for alleviating pain, the method comprising:

(a) producing a genetic construct having nucleic acids encoding a botulinum toxin serotype A;

(b) incorporating the construct into a host cell;

(c) ~~expressing the construct to produce~~ culturing the cell under conditions sufficient for expression of the botulinum toxin serotype A; and

(d) covalently attaching the botulinum toxin serotype A to substance P, wherein H_c has been removed from the botulinum toxin or modified so as to reduce the ability of the botulinum toxin to bind to a receptor for the H_c at a neuromuscular junction.

78. (Currently amended) A method for obtaining an agent for alleviating pain, the method comprising:

(a) producing a genetic construct having nucleic acids encoding a botulinum toxin, wherein the ~~portion~~ nucleotide sequence encoding an H_c of the toxin has been removed;

(b) incorporating the construct into a host cell;

(c) ~~expressing the construct to produce~~ culturing the cell under conditions sufficient for expression of the botulinum toxin; and

(d) covalently attaching the botulinum toxin to substance P.

79-80. (Cancelled)